THE AMERICAN
JOURNAL OF PHARMACY

AUGUST, 1912

CHEMICAL EXAMINATION OF THE BARK OF ERYTHROPHLŒUM GUINEENSE.

By Frederick B. Power and Arthur H. Salway.

A Contribution from the Wellcome Chemical Research Laboratories, London.

The genus *Erythrophlæum* appears to comprise but few species, of which the best known is *E. Guineense,* G. Don of Central and Western Africa. Other species are *E. Couminga,* Baill. from Madagascar; *E. Fordii,* Oliv. from Farther India; *E. chlorostachys* (F. von Mueller), Baill. from Australia, and *E. densiflorum* (Elmer), Merrill, formerly known as *Cynometra densiflora,* Elmer, from the Philippines.[1] In addition to these, Planchon[2] notes that Gagnepain (*Notulæ systematicæ* de Lecomte. Herbier du Muséum de Paris, *t.* ii, fasicule 4, p. 111 *et seq.*) has recently named and described two species which are peculiar to Farther India, namely: *E. cambodanium* and *E. succirubrum.*

The bark of *Erythrophlæum Guineense,* G. Don (Nat. Ord. *Leguminosæ*) is known by a number of common names, such as "sassy bark," "mancona bark," "red-water tree bark," "casca bark," "doom bark," and in the vernacular of the Congo as "Nkasa."[3] It was brought to notice many years ago[4] on account

[1] *Philippine Journal of Science,* 1909, **4,** 267.

[2] *Annales du Musée colonial de Marseille,* 1911, **19,** 6.

[3] The designation "Nkasa" does not seem to be a specific one, since it is also applied to *Strychnos Dewevrei,* Gilg, and possibly to other plants. It has, furthermore, been stated that the expression "Nkasa" does not denote, as has been supposed, the plant from which the ordeal poison has been obtained, but the ordeal itself, and that it is used throughout the Congo ("Compagnie du Kasai. Mission permanente d'Études scientifiques." Résultats de ses Recherches botaniques et agronomiques, mis en order et annotés par E. de Wildeman, Brussels, 1910, p. 211 *et seq.*).

[4] This Journal, 1849, **21,** 97.; 1851, **23,** 301, and 1852, **24,** 195. Also *Pharm. Journ.,* 1856, **16,** pp. 233, 373.

of its intensely poisonous properties, by reason of which it has long been employed by the natives of Western Africa as an ordeal in their trials for witchcraft and sorcery, as well as for other criminal purposes. It also apparently enters into the composition of the arrow poison of the Pigmies.[5] A description of the bark, together with observations respecting its physiological action or that of its constituent alkaloid, has been recorded in various works of reference, such as the "Dispensatories" and other commentaries on the Pharmacopœias, as well as in the periodical literature of pharmacy and medicine. Since these sources of information are so readily available, the present consideration of the literature may chiefly be restricted to some previously published statements respecting the alkaloidal constituent of the bark.

The so-called "Sassy Bark" appears to have been first chemically examined by Gallois and Hardy,[6] who isolated from it a very toxic alkaloid which they designated erythrophleine. This was stated to have been obtained by one method as a transparent, amorphous solid, of a pale amber color, firm consistence, and gummy to the touch, whereas by another method it was yellowish-white, transparent, and had a crystalline appearance, which could be confirmed by the microscope. It was soluble in water and in alcohol, but very slightly soluble in ether or chloroform. The alkaloid was furthermore described as yielding a crystalline hydrochloride and platinichloride, and as giving with potassium permanganate and sulphuric acid a violet color, less intense than that produced by strychnine, and soon changing to a dirty brown. No analysis, melting point, or other specific characters of any of these substances were, however, recorded. The same authors state to have obtained from *Erythrophlæum Couminga* an alkaloid which was believed to be closely related to erythrophleine, if not identical with it.

A pharmacological investigation of erythrophleine was subsequently conducted by Harnack and Zabrocki.[7] The alkaloid employed by them was prepared by a German manufacturer, and it was recorded that the base, as well as its salts and double salts,

---

[5] *Pharm. Journ.*, 1891, [iii], **21**, 917.

[6] *Journ. Pharm. Chim.*, 1876, **24**, 25, and *Bull. Soc. Chim.*, 1876, **26**, 39.

[7] *Arch. f. Exp. Path. Pharm.*, 1882, **15**, 404.

could be obtained only in the form of clear syrups. The substance produced both with cold-blooded and warm-blooded animals the combined effects of digitalin and picrotoxin (clonic spasms). After an interval of several years the investigation was renewed by Harnack,[8] with the use of material which had been procured from the same manufacturer as that previously employed. This specimen of erythrophleine hydrochloride was, however, in the form of a fine, pale yellow, amorphous powder, which, on keeping, agglomerated to a dry, solid mass. In distinction from the alkaloid previously examined, it produced only a digitalin-like effect, and not also that of picrotoxin. The base obtained from this salt was almost completely insoluble in water, but readily soluble in alcohol and in ether, and, contrary to the statement of Gallois and Hardy (*loc. cit.*), showed no tendency to crystallize. From analyses of the platinum salt, the base was considered to possess the formula $C_{28}H_{43}O_7N$ or $C_{28}H_{45}O_7N$, but the difficulty was noted of obtaining preparations which were sufficiently uniform in composition to ensure trustworthy results. On heating with concentrated hydrochloric acid it yielded an amorphous, acidic product, designated erythrophleic acid, $C_{27}H_{40}O_8$ or $C_{27}H_{42}O_8$, and methylamine, $CH_3.NH_2$, whereas the alkaloid first examined, when similarly treated, gave apparently the same acidic substance and a base resembling pyridine, which was termed manconine.

It would seem probable that the varying results obtained in the above-mentioned investigations may be attributed, as Harnack (*loc. cit.*, p. 562) and others have suggested, to the use of barks from different species of *Erythrophlæum*, or possibly from varieties of the same species. This view has recently been corroborated by observations personally communicated to us by Mr. Iner C. Wickware, to whom we are indebted for the material employed in the present investigation and also for photographs from which the illustrations accompanying this paper were produced. The material supplied by Mr. Wickware was designated by him as "Nkasa Bark," but its identity with the so-called "Sassy Bark" (from *Erythrophlæum Guineense*, G. Don) was kindly confirmed by Mr. E. M. Holmes, F.L.S.

The extended use of "Nkasa" bark in the Congo for criminal

[8] *Arch. Pharm.*, 1896, **234**, 561.

purposes, and the great suffering thereby produced, which had been witnessed by Mr. Wickware, led him to bring the bark to our notice, as it was hoped that a study of its constituents would render it possible to suggest a means of obviating the destructive effects of the poison. Inasmuch as the alkaloid erythrophleine had hitherto not been obtained in a form which permitted of its more exact characterization, and the statements concerning it being also somewhat at variance, it was deemed desirable to subject the "Nkasa" bark to a further chemical examination. The results which have now been obtained are summarized at the end of this paper.

EXPERIMENTAL.

The material employed for this investigation was obtained from the Belgian Congo, West Africa, through the kindness of Mr. Iner C. Wickware, of the Christian Missionary Alliance, at Boma, to whom our best thanks may here be expressed. It was collected from living trees, and, as already noted, agreed in its characters with the recorded descriptions of the bark of *Erythrophlæum Guineense*, G. Don.

A small portion (10 grammes) of the ground bark was tested for the presence of an alkaloid with a positive result.

For the purpose of a complete chemical examination 72.9 kilogrammes of the coarsely ground bark were completely extracted by continuous percolation with hot alcohol. After the removal of the greater portion of the alcohol, 27.68 kilogrammes of a dark red, viscid extract were obtained.

A quantity (4.5 kilogrammes) of the above-mentioned extract was mixed with water and distilled in a current of steam, but it yielded no essential oil. After this operation there remained in the distillation flask a deep red, aqueous liquid (A) and a quantity of a viscid resin (B), which solidified on cooling. The resin was thoroughly washed with water, and the washings added to the main portion of the aqueous liquid.

Examination of the Aqueous Liquid (A).

The aqueous liquid was concentrated under diminished pressure, and then repeatedly extracted with ether. The ethereal liquid was washed, dried, and the solvent removed, when 12 grammes of a brown, varnish-like solid were obtained.

Isolation of Luteolin, $C_{15}H_{10}O_6$.

The above-mentioned ethereal extract was redissolved in ether, and the solution shaken first with aqueous ammonium carbonate, which, however, extracted but a small amount of amorphous, gummy material. It was then shaken with successive portions of aqueous sodium carbonate until nothing further was removed by this reagent. The sodium carbonate extracts were united and acidified, when a lemon-yellow, crystalline solid was precipitated. This was collected and recrystallized several times from dilute alcohol, when it was obtained in pale yellow, glistening needles, which melted and decomposed at 323°. The amount of pure substance thus isolated from 4.5 kilogrammes of the original alcoholic extract was 0.2 gramme, and therefore represented 0.0017 per cent. of the weight of the bark. On subsequently treating 12 kilogrammes of the extract in the manner already described, a further quantity (0.5 gramme) of the yellow, crystalline substance was obtained.

0.1012* gave 0.2338 CO_2 and 0.0350 H_2O. C = 63.0; H = 3.8.
0.0898* gave 0.2072 CO_2 and 0.0312 H_2O. C = 62.9; H = 3.9.
$C_{15}H_{10}O_6$ requires C = 62.9; H = 3.5 per cent.

The substance was readily soluble in aqueous sodium carbonate and sodium hydroxide, forming deep yellow liquids, whilst its alcoholic solution gave with ferric chloride an intense green color. It dissolved in concentrated sulphuric acid, yielding a yellow solution which gradually develops a green fluorescence. On heating the substance for some time with acetic anhydride, and subsequently removing the greater portion of the latter, a compound separated, which, when recrystallized from ethyl acetate, was obtained in fine, colorless needles, melting at 222°–224°. This compound was likewise analyzed.

0.1038 gave 0.2320 CO_2 and 0.0384 H_2O. C = 60.9; H = 4.1.
$C_{15}H_6O_6(CO.CH_3)_4$ requires C = 60.8; H = 4.0 per cent.

From the above results it may be concluded that the yellow substance possesses the empirical formula $C_{15}H_{10}O_6$, and that it contains four hydroxyl groups. These facts, together with the above-described characters, render it evident that the substance is

* Dried at 120°.

luteolin, a tetrahydroxyflavone, which was first isolated from the so-called Dyer's Weed (*Reseda luteola,* Linné), and which also occurs in the leaves of *Digitalis purpurea,* Linné, being identical with the compound termed digitoflavone.[9]

The ethereal liquid from which the luteolin had been removed by extraction with sodium carbonate, as above described, was next shaken with a solution of sodium hydroxide, which, however, removed practically nothing. On finally drying and evaporating the ethereal liquid, it yielded about 2 grammes of amorphous material, from which nothing definite could be obtained.

The deep red aqueous liquid which had been completely extracted with ether was subsequently shaken with successive portions of warm amyl alcohol, these extracts being then united, washed with a little water, and concentrated to a small volume under diminished pressure. The concentrated amyl alcohol extracts deposited nothing on keeping, but on the addition of toluene an amorphous, nearly colorless precipitate was formed, which, when collected and dried on a porous tile, amounted to 25 grammes. This product was extremely soluble in water, giving a deep red solution, from which sodium carbonate precipitated a brown, amorphous solid, soluble in an excess of the reagent. The solid substance was found to contain traces of nitrogen, but nothing definite could be isolated from it. In order to ascertain whether it was glucosidic in character, a portion was heated for some time with 5 per cent. sulphuric acid. During this operation a large amount of resinous matter separated, which was subsequently removed by filtration, and the clear, acid liquid then extracted with ether. The ethereal liquid yielded, besides some amorphous material, a very small amount of a crystalline substance, which was identified by its melting point and reactions as gallic acid. The aqueous, acid liquid was finally deprived of sulphuric acid by means of baryta, concentrated, and examined for sugar, but with a negative result. It was therefore evident that the above-described solid was not glucosidic.

The original aqueous liquid (A), after having been extracted with ether and amyl alcohol as described above, still retained a deep red color, and gave slight precipitates with the usual alkaloid reagents. A small portion of the liquid was treated with basic lead acetate, which produced an abundant, dark colored precipitate, and

[9] *Ber. d. deutsch. chem. Ges.,* 1899, **32,** 1184.

the mixture filtered. The filtrate was deprived of lead by means of hydrogen sulphide, again filtered, and concentrated to a small volume. It was found to contain a considerable quantity of sugar, which yielded an osazone melting and decomposing at 210°. The basic lead acetate precipitate was suspended in water, decomposed by hydrogen sulphide, and the mixture filtered. The resulting liquid evidently contained a large amount of tannin, and, although it gave precipitates with the alkaloid reagents and with sodium carbonate, nothing definite could be isolated from it. There was no indication of the presence of a glucoside.

The main portion of the aqueous liquid was carefully neutralized with sodium carbonate, when a brown, amorphous precipitate was deposited, which, after being collected, washed with water, and dried, amounted to about 15 grammes. This product contained nitrogen, but it was very indefinite in character. It was insoluble in water and dilute mineral acids, as also in ether or chloroform, and was only sparingly soluble in alcohol. It dissolved readily in alkalies, giving a deep brown solution. When warmed with dilute hydrochloric acid, the liquid gave no reaction with alkaloid reagents. All attempts to prepare derivatives from it led only to the formation of resinous substances.

Isolation of the Alkaloid, Erythrophleine.

The aqueous liquid from which the above-mentioned brown, amorphous product had been separated, was made strongly alkaline with sodium carbonate and repeatedly extracted with ether. These extracts were united, washed with a little water, then dried, and a current of dry hydrogen chloride passed into the ethereal liquid. The alkaloid present was thus precipitated as a viscid hydrochloride, from which the ether was removed by decantation. The hydrochloride, amounting to about 1 gramme, was dissolved in a little alcohol, and ethyl acetate added, but no precipitation ensued. On subsequently evaporating the liquid to dryness in a vacuum desiccator over sulphuric acid, the salt was obtained in the form of a brown, scaly solid, which was soluble in water and responded to the tests for an alkaloid with the usual reagents. In order to further purify this product, it was redissolved in water, and aqueous sodium carbonate cautiously added until the precipitation of the base was complete. The mixture was then extracted with ether, filtered to remove some insoluble resinous matter, and the ethereal

liquid again treated with dry hydrogen chloride. The gummy hydrochloride thus obtained was dissolved in ethyl acetate containing some alcohol, and the solution then evaporated at the ordinary temperature under diminished pressure over sulphuric acid. In this manner the alkaloidal salt was obtained as an almost colorless, amorphous solid, amounting to 0.6 gramme. It was readily soluble in water or alcohol, and was extremely hygroscopic, becoming gradually converted into a brown, transparent, glutinous mass. The free base, which has been termed erythrophleine by previous investigators, was a colorless, amorphous solid, readily soluble in ether, ethyl acetate, or alcohol, but insoluble in water. It yielded a bright yellow picrate, and an almost colorless gold salt, but neither of these derivatives could be obtained in a crystalline state.

The properties of erythrophleine, as described above, do not entirely agree with those recorded by Gallois and Hardy (*loc. cit.*), but are more in accordance with the later observations of Harnack.[10] A commercial specimen of erythrophleine hydrochloride, which was obtained by us from the same source as the product last employed by Harnack, was also similar in character to that described by him. It was a nearly colorless, amorphous powder, which on keeping in a closed tube gradually became converted into a brown, gummy mass. It dissolved readily in water, yielding a colorless solution, which was neutral to litmus and responded to the usual alkaloidal tests. On the addition of aqueous sodium carbonate the free base was precipitated as a gummy solid, which was insoluble in water or alkalies, but readily soluble in ether. The dry ethereal solution of the base, when treated with dry hydrogen chloride, yielded the hydrochloride in the form of a viscid oil and not in the original condition as an amorphous solid. Both the picrate and the aurichloride of the base were likewise amorphous. The amount of chlorine in this commercial specimen of erythrophleine hydrochloride was determined with the following result:

0.1652 gave 0.0462 AgCl. Cl = 6.9.
$C_{28}H_{43}O_7N.HCl$ requires Cl = 6.6 per cent.

Although the result of this determination is in approximate agreement with the formula assigned to erythrophleine by Harnack,

[10] *Arch. Pharm.*, 1896, **234**, 561.

it is evident that the substance is of too indefinite a character to permit of any deduction respecting its ultimate composition.

The aqueous liquid from which the erythrophleine had been removed, as above described, was made strongly alkaline with sodium hydroxide and extracted with various solvents, but no further quantity of alkaloidal substance was thus obtained. The proportion of alkaloid in the drug was therefore extremely small, since the amount of hydrochloride originally obtained (about 1 gramme) represented not more than 0.008 per cent. of the weight of bark employed.

Examination of the Resin (B).

The resinous material obtained from the original alcoholic extract of the bark, as previously described, formed, when dry, a dark brown, brittle mass, which could be reduced to a powder. It amounted to 1600 grammes, and thus represented 13.5 per cent. of the weight of the bark. For the purpose of its examination, 500 grammes of the resin were dissolved in alcohol, the solution mixed with purified sawdust, and the thoroughly dried mixture successively extracted in a Soxhlet apparatus with various solvents, when the following amounts of extracts, dried at 100°, were obtained:

Petroleum (b. p. 30–45°)	extracted	35.5	grammes =	7.1	per cent.
Ether	"	26.0	" =	5.2	" "
Chloroform	"	3.5	" =	0.7	" "
Ethyl Acetate	"	26.0	" =	5.2	" "
Alcohol	"	400.0	" =	80.0	" "
	Total	491.0	grammes =	98.2	per cent.

Petroleum Extract of the Resin.

This extract was a dark brown, soft, fatty solid. It was tested for the presence of an alkaloid, but with a negative result. The whole of the extract was then hydrolyzed by heating for a short time with an alcoholic solution of potassium hydroxide, after which the alcohol was removed, water added, and the alkaline mixture repeatedly extracted with ether. The ethereal extracts were united, washed, dried, and the solvent removed, when a quantity (20 grammes) of a reddish-brown, viscid solid was obtained.

Isolation of a Phytosterol, $C_{27}H_{46}O$.

The above-mentioned unsaponifiable material was distilled under diminished pressure, when a small quantity passed over below 260°/10 mm., but the greater portion distilled with slight decomposition above 300°/10 mm., a small final fraction being separately collected. The principal portion did not crystallize on cooling, but formed a viscid, transparent, yellow gum. A little of this substance, when dissolved in a mixture of chloroform and acetic anhydride, and a drop of sulphuric acid subsequently added, gave a reddish-brown coloration, rapidly changing to green, thus indicating the presence of a phytosterol. The gummy substance was therefore dissolved in hot alcohol, and the liquid cooled with vigorous agitation, when a colorless solid was gradually deposited. This was collected and recrystallized from alcohol, from which it separated in colorless, glistening leaflets, melting at 130–133°.

0.2378, on heating at 110°, lost 0.0112 H_2O. $H_2O = 4.7$.
0.1334 * gave 0.4110 CO_2 and 0.1459 H_2O. $C = 84.0$; $H = 12.1$.

$C_{27}H_{46}O$, H_2O requires $H_2O = 4.5$ per cent.
$C_{27}H_{46}O$ requires $C = 83.9$; $H = 11.9$ per cent.

The above-described substance was evidently a phytosterol, and it gave the color reactions of that class of compounds.

The small final fraction obtained by the distillation of the unsaponifiable material yielded a phytosterol which crystallized in leaflets melting somewhat indefinitely between 135° and 142°. It would thus appear that more than one phytosterol was present in the distilled product, but the amount of substance was too small to permit of a more complete separation.

Examination of the Fatty Acids.

Isolation of Cerotic Acid, $C_{26}H_{52}O_2$.

The alkaline, aqueous solution of potassium salts, which had been extracted with ether as above described, was acidified with dilute sulphuric acid, and the precipitated fatty acids dissolved in ether. The ethereal solution was washed, dried, and the solvent removed, when a semi-solid residue was obtained, amounting to 12

* Anhydrous substance.

grammes. This was distilled under diminished pressure, and two fractions collected, which passed over at 200–250°/12 mm., and above 250°/12 mm., respectively. The latter fraction rapidly solidified, and was purified by crystallization from ethyl acetate, when an acid was obtained which separated in minute, colorless needles, melting at 76–77°.

0.0657 gave 0.1895 CO_2 and 0.0796 H_2O. C = 78.7; H = 13.4.
$C_{26}H_{52}O_2$ requires C = 78.8; H = 13.1 per cent.

This substance was thus identified as cerotic acid.

The fraction of fatty acids distilling at 200–250°/12 mm. amounted to 6 grammes, and was evidently a mixture of saturated and unsaturated acids. It was therefore converted into the lead salt, and the latter treated with ether. The portion of lead salt which was insoluble in that solvent yielded the saturated acids, which, after recrystallization from alcohol, melted at 48–50°.

0.4208 required for neutralization 15.7 c.c. $\frac{N}{10}$ KOH. N.V. = 209.3.
$C_{16}H_{32}O_2$ requires neutralization value = 219.1.
$C_{18}H_{36}O_2$ requires neutralization value = 197.5.

It is thus evident that the saturated acids present in the above-mentioned fraction consisted of a mixture of palmitic and stearic acids, and apparently in about equal proportions.

The portion of lead salt which was soluble in ether yielded 3.5 grammes of unsaturated acids, which distilled at 215–225°/10 mm. These were analyzed, and the neutralization and iodine values determined, with the following results:

0.1753 gave 0.4950 CO_2 and 0.1836 H_2O. C = 77.0; H = 11.6.
0.5902 required for neutralization 20.2 c.c. $\frac{N}{10}$ KOH. N.V. = 192.0.
0.1096 absorbed 0.1449 iodine. Iodine value = 132.2.
$C_{18}H_{34}O_2$ requires C = 76.6; H = 12.1 per cent.
Neutralization value = 198.9; Iodine value = 90.1.
$C_{18}H_{32}O_2$ requires C = 77.1; H = 11.4 per cent.
Neutralization value = 200.4; Iodine value = 181.4.

It would appear from these results that the unsaturated acids consisted of a mixture of oleic and linolic acids.

Ether Extract of the Resin.

This extract was a brown, brittle solid, amounting to 26 grammes. It was digested with ether, when the greater portion passed into solution, while a small amount of a colorless powder remained undissolved. The latter was very sparingly soluble in alcohol, and when dissolved in chloroform, a little acetic anhydride added, and subsequently a drop of concentrated sulphuric acid, it gave a pink coloration, rapidly changing to blue and green. It also yielded an acetyl derivative, which separated in colorless leaflets, melting at 162–163°. From these characters it may be concluded that the sparingly soluble substance was the dihydric alcohol, ipuranol, $C_{23}H_{38}O_2(OH)_2$, but the amount obtained was not sufficient for analysis.

The above-mentioned ethereal liquid was shaken successively with dilute hydrochloric acid and aqueous solutions of ammonium carbonate, sodium carbonate, and sodium hydroxide. The hydrochloric acid removed only traces of a gummy base, which gave reactions with the usual alkaloid reagents, whilst the ammonium carbonate caused the precipitation of a small amount of a resinous solid of indefinite character. The sodium carbonate extract yielded, on acidification, a pale yellow solid. This was collected, and crystallized from dilute alcohol, when it separated in pale yellow needles, and gave a colorless acetyl derivative melting at 222–224°. It was thus found to be identical with luteolin, which had previously been isolated, as above described.

The final extraction of the ethereal liquid with sodium hydroxide yielded nothing but amorphous products, and on subsequently evaporating the ether only traces of a soft resin remained.

Chloroform, Ethyl Acetate and Alcohol Extracts of the Resin.

The chloroform extract of the resin was a dark brown, viscid solid, amounting to only 3.5 grammes, and nothing was isolated from it.

The ethyl acetate extract of the resin was a brown, viscid solid, which amounted to 26 grammes. In order to ascertain whether it contained anything glucosidic, it was heated for some time with a 5 per cent. solution of sulphuric acid in aqueous alcohol. On subsequently distilling the mixture with steam, no volatile oil or

acid passed over. The distillation flask then contained, besides the aqueous liquid, a quantity of a viscid resin, which was collected, dissolved in alcohol, dried on purified sawdust, and the mixture extracted with ether in a Soxhlet apparatus. From this ethereal extract a small amount of a yellow, crystalline substance was obtained which proved to be identical with the previously described luteolin.

The aqueous, acid liquid, from which the above-mentioned viscid resin had been separated, was first extracted with ether, which, however, removed only a small quantity of gummy material. It was then freed from sulphuric acid by means of barium hydroxide, and finally concentrated under diminished pressure to a small volume. This liquid readily reduced Fehling's solution, and yielded an osazone melting and decomposing at 210°.

The results above described indicated the presence of a glucoside in the ethyl acetate extract of the resin, and it is probable that the small amount of luteolin which was isolated after the treatment with dilute sulphuric acid represented one of its hydrolytic products.

The alcohol extract of the resin was a brownish-black, brittle solid, which amounted to 400 grammes, and represented 80 per cent. of the total resinous material employed. It was completely amorphous, and as it appeared to undergo no change when heated with dilute sulphuric acid in aqueous alcohol, it evidently contained nothing of a glucosidic character.

Physiological Tests.

In order to determine the action of the extract prepared from *Erythrophlæum* bark, and of some of the products obtained therefrom, a number of tests were kindly conducted for us by Dr. H. H. Dale, Director of the Wellcome Physiological Research Laboratories, to whom our best thanks may here be expressed.

One gramme of the original alcoholic extract, representing about 2.6 grammes of the bark, was administered to a dog, when it caused continuous vomiting and a marked slowing of the heart-beat. One gramme of the total resinous material (= 7.4 grammes of bark) had much less effect, but caused some vomiting. A quantity of the water-soluble portion of the original alcoholic extract, representing 4 grammes of the bark, caused retching, without actual vomiting, and a distinct action on the heart.

The previously described amyl-alcohol extract of the aqueous liquid (A) was tested by dissolving 0.07 gramme of the respective extract in water, and injecting this intravenously into a rabbit, but it had no perceptible effect. The amount of substance employed represented about 13.6 grammes of the bark.

The brown, amorphous substance which was precipitated from the aqueous liquid (A) by its neutralization with sodium carbonate, as previously described, could not be tested by intravenous injection on account of its insolubility. A small quantity (0.25 gramme) of the product, representing about 216 grammes of the bark, was therefore given to a dog by the mouth, but no perceptible effect was produced.

The alkaloid erythrophleine, which was tested in the form of its hydrochloride, produced results similar to those obtained from a commercial specimen of this salt, which was examined for the purpose of comparison, and in both cases the action was such as has been described by previous observers. When given intravenously to a rabbit, 0.001 gramme of commercial erythrophleine hydrochloride caused marked slowing of the heart-beat, whereas with 0.002 gramme the same effect was followed by secondary quickening of the heart-beat, paresis, trismus, and finally death in convulsions in about half an hour. Similar results were obtained from the alkaloid isolated in the course of the present investigation, and 0.005 gramme of the hydrochloride of this base killed a small rabbit in less than five minutes. Both preparations of the alkaloid, when injected intravenously into pithed cats in doses of 0.0025 gramme, caused quite typical digitalis-like effects, the rise of blood pressure being large, and the heart passing through delirium to permanent systole.

Since the precipitate obtained by treating a small quantity of the water-soluble portion of the original alcoholic extract with basic lead acetate, when suspended in water and decomposed by hydrogen sulphide, yielded a liquid which possessed in a marked degree the characteristic erythrophleine action, it would appear that an appreciable amount of active substance had been precipitated by the lead salt. As already noted, however, nothing definite could be obtained from the lead compound, and for the purpose of isolating the alkaloid from the main portion of the original aqueous liquid (A) the latter was not subjected to treatment with basic lead acetate.

Summary.

The results of the present investigation of the bark of *Erythrophlæum Guineense,* G. Don, and the deductions therefrom, may briefly be summarized as follows:

A quantity of the bark was completely extracted with hot alcohol, and the resulting concentrated extract distilled in a current of steam, but it yielded no essential oil.

From the portion of the extract which was soluble in water the following substances were isolated: a very small amount of luteolin, $C_{15}H_{10}O_6$, and a small amount of an alkaloid which agreed in its characters and physiological action with erythrophleine, as described by Harnack (*Arch. Pharm.,* 1896, **234**, 561). Neither the alkaloid nor its salts could be obtained in a crystalline state, and they were therefore not considered suitable for analysis. The aqueous liquid contained, furthermore, besides some indefinite amorphous material, a considerable quantity of tannin, and a sugar which yielded *d*-phenylglucosazone, melting at 210°.

The portion of the alcoholic extract which was insoluble in water consisted of a dark brown, brittle resin, and represented 13.5 per cent. of the weight of the bark. From this product the following substances were obtained: a phytosterol, $C_{27}H_{46}O$ (m. p. 130–133°); cerotic, stearic, palmitic, oleic and linolic acids; and very small amounts of ipuranol, $C_{23}H_{38}O_2(OH)_2$, and luteolin, $C_{15}H_{10}O_6$. A portion of the latter compound was apparently contained in the resin in the form of a glucoside.

Inasmuch as the results of a preliminary test had indicated a much larger proportion of alkaloid to be contained in the bark than could subsequently be isolated, it appears probable that some change had taken place during the processes of extraction. This could not be more precisely determined on account of the very indefinite character of the base, which also precluded its further chemical study.

Since the bark under consideration is an exceedingly violent poison, and is largely used in West Africa for criminal purposes, it may finally be noted that the recognized and apparently most efficient antidote consists in the prompt administration of an emetic, or use of the stomach-pump, with subsequent stimulant remedies.

CUDBEAR AS A PHARMACEUTICAL COLORING.*

By George M. Beringer.

Since 1874, when Hans Wilder[1] directed attention to the advantages of cudbear over cochineal and carmine as a coloring agent for pharmaceutical preparations, it has become very popular and is now extensively used. As a vegetable dye miscible with either slightly acid or alkaline solutions with the production of acceptable shades of red, it has been used by many almost as a universal coloring where red colored liquids are wanted. Despite its extensive use and desirable tinctorial properties, one has but to note the criticisms in the pharmaceutical press to learn that it has not proved entirely satisfactory.

The principal complaint has been the lack of uniformity in the color of preparations as made by formulas in which the tincture of cudbear was directed. This tincture as found in the drug stores is exceedingly variable, due in part to the variability of commercial cudbear and in a large measure to the imperfect extraction of cudbear by the official N. F. formula.

This tincture is directed in a number of the National Formulary recipes, and in the revision now in progress it has again been decided to retain cudbear as a coloring agent. The desirability of adopting a method of standardizing the tincture is obvious and a sub-committee on color standards have been giving earnest consideration to this vexing problem.

A few of the suggestions offered for this purpose may be here mentioned. One of the earlier thoughts was the publication of a color chart with the shades designated by numbers and to indicate in the formula for a preparation the number of the shade that the product should match. A similar suggestion was to color silk thread or woolen yarn to the desired shades and chart and number these as guides. Tinted glass, especially that known as "ruby flash glass," was recommended for comparing acid solutions of cudbear. Tintometers were recommended, but these are beyond the reach of

* Presented at the meeting of the New Jersey Pharmaceutical Association, Atlantic City, N. J., June 5, 1912.

[1] American Journal of Pharmacy, 1874, 299.

the average pharmacist and so not practicable. A novel proposition along this line was offered by Harvey I. Leith,[2] namely, that standard glass rods be prepared of definite diameter and length and colored in their manufacture according to standards established by the Committee on National Formulary, each rod to have a groove at the top bearing a tag with a number indicating the color. A rod of the standard tint dipped into a preparation would not be discerned if the coloring matched; if the rod showed it would indicate that the preparation was off in color. None of these suggestions were found to be practicable. The question naturally arises as to the proper directions that would have to be given in such schemes to insure the manipulator matching the shade.

A more promising line of work has been the attempt to standardize dilutions of cudbear tinctures against chemical solutions of definite strength and color such as solution of iodine, solution of gold tribromide or an alkalized phenolphthalein solution. The latter, a suggestion of Mr. Otto Raubenheimer, has been more favorably considered by the Committee, and will be more particularly referred to later on. Other methods suggested have been chemical, such as the determination of the tinctorial value by the amount of sulphurous acid or solution of sodium hypochlorite required to bleach out a specified volume of the tincture previously well diluted.

Chairman Diehl[3] is undoubtedly correct in his opinion "that any other than the simplest method of standardization will prove disastrous since it is not likely to be carried out by the average pharmacist." His suggestion was[4] "that comparison of the diluted tincture should be made with dilutions of the purest attainable form of the real active coloring principles." Prof. H. V. Arny[5] states that "orcein, the active principle of cudbear, was found to be of uniform tinctorial power and a solution of orcein 1 to 40,000 was found to match a dilution 1 to 100 of a sample of tincture of cudbear."

If a purified cudbear or a satisfactory extract thereof of a uniform quality could be readily prepared, then the problem of standard red colored solutions would be simplified. Still better would be

[2] *American Druggist*, 1910, 175.
[3] A. Ph. A. Bulletin, 1909, 379.
[4] A. Ph. A. Bulletin, 1910, 371.
[5] A. Ph. A. Bulletin, 1910, 371; also *Practical Druggist*, 1912, 24.

the isolation of the coloring principles in a reasonably pure condition. A tincture made with a definite proportion of such extract or coloring principle should not vary greatly and only slight difference of shades could exist in the preparations colored therewith. In the investigations of the writer, he has endeavored to keep this thought continuously in mind and his experiments have been largely directed thereby.

Cudbear is one of the interesting group of dyes, orchil, cudbear and litmus that are commercially produced from various lichens. Lichens contain in their tissues a number of different coloring principles and E. Bachmann,[6] who studied this subject, distinguished by micro-chemical reactions sixteen different pigments, including greens, yellows, browns and reds of various shades. Moreover, various lichens contain colorless acids and ester-like compounds of orcin and closely related substances which through the action of alkalies, air and water, split to produce first *orcin,* which is itself colorless, but by the continued action of ammonia and air is converted into *orcein* and other colored substances. In former times, the ammonia needed was supplied by the use of stale urine. It is to be hoped that, with the modern methods of obtaining ammonia, this disgusting process is no longer practiced; yet the odor obtained from some samples seem to indicate the possibility of its continuance. By the use of different species of lichens and modification of the process and of the alkali used, different end products are produced resulting in the commercial dyes named.

Cudbear is stated to be prepared principally from certain species of Lecanora and Variolaria. Lecanora tartarea of northern Europe is said to be the source of most of that in the market and hence this species has been named the cudbear plant or cudweed. The name cudbear was given to this dye in honor of Dr. Cuthbert Gordon, who introduced it as a dye into Great Britain in the latter half of the eighteenth century. As a dye it is indifferent to cotton but valuable in the dyeing of wool and silk.

As a commercial product it is very prone to adulteration and in pharmacy its use should be restricted to a selected article that has been carefully tested and found to comply with the standard adopted. While good cudbear yields an ash of from 5 to 12 per

[6] *Pringsheim's Jahrbucher,* vol. xxi, p. 1.

cent., some samples examined gave an ash equivalent to 30 per cent. This consists very largely of sodium chloride which is a common adulterant and which, according to Allen,[7] " is sometimes added to reduce an unusually rich article to a uniform standard of quality." In one sample the writer found such an abundance of salt present that a portion crystallized out on evaporating the ammoniacal solution as directed in Hankey's process for tincture of cudbear.

As the coloring of cudbear is only slightly soluble in cold water I strongly advocate that all cudbear used for pharmaceutical purposes be first washed with at least five times its weight of cold water. On mixing cudbear with this amount of cold water and allowing it to macerate for a few hours with occasional agitation before filtering, the aqueous solution removes most of the sodium chloride, some ammonium salts with their empyreumatic odor, as well as some organic products that are undesirable, such as undecomposed lichen acids, partly converted orcin and extractive. The washed cudbear is more readily extracted and loses scarcely any of its real tinctorial power. This is a simple refinement that should be introduced no matter what formula be adopted for the preparation of a standard tincture.

Tincture of Cudbear and Its Standardization.

It is impossible to entirely exhaust cudbear of its coloring, and in the preparation of the tincture it is safe to assert that the amount directed in the N. F. formula for Tinctura Persionis, 125 gms. in the liter, is not half extracted and that an equally satisfactory preparation would result if the cudbear be reduced one-half and hereafter a tincture of not over 10 per cent. drug strength should be directed.

The directions for the manipulation in the N. F. read exceedingly simple: " Pack the Cudbear in a suitable percolator, and percolate it with a mixture of one (1) volume Alcohol and two (2) volumes of Water until 1000 c.c. of Tincture are obtained."

Cudbear is a most troublesome substance to percolate. It is difficult to moisten evenly and not infrequently a portion becomes pasty and other portions scarcely moistened, with the result that one is prone to obtain either channelling and uneven extraction or more frequently clogging of the percolator which may even com-

[7] Commercial Organic Analysis, vol. iii, pt. 1, p. 324.

pletely stop the process. Consequently, if percolation is to be adopted, the plan of admixing with the cudbear an inert diluent must be resorted to. A number of substances, such as sand and ground pumice, which usually serve for this purpose with other refractory drugs, have not proved satisfactory with cudbear. In my experience ground cork has proved entirely satisfactory, about one-third to one-half of the weight of the cudbear being sufficient. The commercial ground cork of the factories, however, should not be used as this is made from the cork refuse and siftings and is full of foreign matters and dirt and has a musty odor. Unsightly corks, or even old corks, if thoroughly cleansed by boiling with water and drying, will serve the purpose and these can readily be reduced to a powder passing through a No. 20 to a No. 40 sieve by the use of an almond grater, a useful implement that should be in every drug store and laboratory.

The official menstruum is likewise too weak in alcohol to serve as a solvent for the coloring matters present. Water alone is a poor solvent for these and alcohol is one of the best, and if the menstruum is to be hydro-alcoholic then a mixture of, at least, alcohol 3 volumes water 1 volume is to be recommended. The coloring principles present in cudbear are, at least in part, associated as ammonia compounds and some are not soluble even in alcohol until alkali be added. Orcein, the most important dye constituent of cudbear, as found in the market, is but indifferently soluble in alcohol and its color is not fully developed unless ammonia or other alkali be present. Hence, ammoniacal extraction of cudbear seems to be indicated, and, when the extraction is so made then the alcohol can be reduced to the amount needed as a preservative. This is the principle that has been followed in the formula for tincture proposed by Wm. T. Hankey.[8] His formula is cudbear 125 gms., macerate for 36 hours in a mixture of ammonia water 125 c.c. and water 1875 c.c., shaking at intervals, then filter and wash the residue on the filter with water until 2000 c.c. of filtrate is obtained. Evaporate the filtrate to 500 c.c., then add 330 c.c. of alcohol and sufficient water to obtain 1000 c.c. of tincture. This is a decided improvement on the present N. F. formula, but the extraction of the cudbear is far from complete, the ammonia directed being insufficient for this purpose.

[8] A. Ph. A. Bulletin, 1908, p. 93.

Moreover, tincture made by this formula shows a tendency to precipitate.

It has been proposed to adopt the Hankey formula in this revision of the National Formulary [9] with the modification that the tincture is to be standardized by the method proposed by Mr. Raubenheimer. The directions being that 1 c.c. of the concentrated ammoniacal extract mixed with 399 c.c. of water and 1 drop of 1 per cent. solution of ammonia should match the color of the Standard Pink Phenolphthalein Solution. The Standard Pink Solution is to be prepared by mixing 1 c.c. of Phenolphthalein T. S. U.S.P. with 2 drops of Solution of Potassium Hydroxide and sufficient distilled water to make 100 c.c.

If these match, the dilution with alcohol and water is carried out as directed in the formula. If they do not correspond, then the amount of the cudbear solution required to match the standard is determined by a repetition of the color comparison and the proper degree of dilution or concentration is thus fixed. The standard tincture should be of such a strength that 1 c.c. diluted with 199 of water should match the standard pink phenolphthalein solution.

The writer experimented with standard color test solutions as proposed by Raubenheimer [10] and also with the solution of orcein 1 to 40,000 as proposed by Arny.[11] The latter had a more decided red tint than the dilution of the cudbear tincture prepared by the proposed formula and was more difficult to match. With some of the samples of tincture made by other formulas the dilution did not match the tints of either of these proposed standards. It is apparent that while the amount of alkali directed to be added to the cudbear dilution (1 drop of 1 per cent. solution of ammonia) may be sufficient to develop the purplish tint desired in a tincture made by ammoniacal extraction, it would be inadequate for such a purpose in tinctures made by other formulas. This was all the more evident in the stronger tinctures resulting from my experiments. In some of these a nearer approach to an exact matching of tints was obtained by a modification of Raubenheimer's standard made by increasing the alkali and diminishing the phenolphthalein; thus 1 c.c. of solution of potassium hydroxide, .2 c.c. of phenolphthalein T.S. and dis-

[9] Bulletin of N. F. Committee No. 31, p. 363.
[10] Bulletin Committee on N. F. No. 31, p. 364.
[11] *The Practical Druggist*, 1912, April, p. 24.

tilled water sufficient to make 100 c.c. Using all three of these solutions for comparison, it was found difficult to accurately match some of the dilutions of the tincture. Comparisons were more readily made, however, if the standards were diluted to 200 c.c. or one-half of proposed strength.

While these standards may serve the purpose for the comparison of tinctures made by the proposed N. F. formula, they are not entirely satisfactory and they are of questionable value in determining the strength of cudbear tinctures made by other processes. In some of these, the directions given must be deviated from, at least by the addition of more ammonia, and then it becomes difficult to determine in each case the exact amount of alkali that is required to produce the purplish pink tint to match that of the standard. An excess of alkali will produce a deep purple not at all comparable with the standards that have been proposed.

In order to make comparison of different tinctures, the writer surmised that if sufficient alkali be added to the diluted tinctures to produce the full purple coloration then the strength of such dilution should be readily and more accurately estimated. Such a method would necessitate the selection of a standard purple solution of known value. It was hoped that the Arny Orcein solution of 1–40,000 upon the addition of alkali would serve the purpose, but on trial it was found that the shade of purple it produced varied too greatly from that of cudbear solutions to be at all satisfactory. This is explained by the fact, as will be shown later, that the other color substances associated with orcein in cudbear materially influence the colors produced by the tincture and that they cannot be ignored. It is possible that a dilute solution of potassium permanganate may serve for such standard.

From the cudbear selected for the experiments, a number of samples of tincture of cudbear were prepared by different formulas. In all of these 125 gms. of the Cudbear was used to the liter of tincture so as to make the comparison with the formula of the N. F. III fair. The cudbear was previously washed with 5 times its weight of water before extraction and ground cork used as the diluent. The products were tested by determining the amount necessary to be diluted to 100 c.c. to match as near as possible the proposed standards. Subsequently, a practical test was also added, the determination of the amount of each necessary to color 100 c.c. of Aromatic Elixir, U.S.P., to a uniform color; 1 c.c. of the sample

made by the N. F. III formula being used as the basis of this test. The following tabulated statement shows the results:

No.	Formula.	Arny Orcein Standard.	Raubenheimer Standard Phenolphthalein Pink Solution	Beringer Modification	To color 100 c.c. Aromatic Elixir.
1	National Formulary III	3 c.c. after adding 3 drops NH_3 (1%)	2.4 c.c. after adding 5 drops NH_3 (1%)	2.2 c.c after adding 5 drops NH_3 (1%)	1. c.c.
2	Hankey's Recipe	2 c.c. after adding 2 drops NH_3 (1%)	1.7 c.c. after adding 3 drops NH_3 (1%)	1.5 c.c. after adding 2 drops NH_3 (1%)	.8 c.c.
3	Menstruum—Diluted Alcohol	1 c.c. after adding 2 drops NH_3 (1%)	.8 c.c. after adding 2 drops NH_3 (1%)	.6 c.c. after adding 4 drops NH_3 (1%)	.3 c.c.
4	Menstruum—Alcohol 3 vols. Water 1 vol.	.8 c.c. Tint not well matched	.5 c.c. after adding 2 drops NH_3 (1%). Difficult to match exactly.	.5 c.c. after adding 2 drops NH_3 (1%) not exactly matched.	.2 c.c.
5	Menstruum—Alcohol	1. c.c. Dilution cloudy and difficult to match exactly by addition of NH_3	.65 c.c.	.65 c.c.	.22 c.c.
6	Menstruum—Stronger Ammonia Water 25 c.c. Alcohol 975 c.c. Finish with alcohol	1.2 c.c. Dilution too purple and comparisons approximate only	.8 c.c.	.9 c.c.	.25 c.c. Not same tint.
7	Menstruum—Ammonia Water U. S. P. 1 vol. Water 3 vols. Percolate 4000 c.c. Evaporate to 750 c.c., when cold add alcohol 250 c.c. and water qs. to make 1000 c.c.	.8 c.c.	.57 c.c. good match	.6 c.c. good match	.2 c.c.
8	Mix the cudbear with Hydrochloric Acid 25 c.c., allow to dry and then percolate with alcohol to 1000 c.c.	.5 c.c. after adding 4 drops NH_3 (1%)	.5 c.c. after adding 4 drops NH_3 (1%)	.5 c.c. after adding 4 drops NH_3 (1%)	.2 c.c.

While these experiments do not permit of the exactness of determinations made by the methods of chemical analysis, they are nevertheless sufficiently instructive to permit of the following deductions: The present N. F. formula gives the poorest preparation, for the reasons explained. The formula proposed for the revision is not the best that can be devised and does not extract the cudbear nearly as thoroughly as can be done by percolation with ammonia

water as in formula No. 7. If the N. F. is to adopt a formula in which the extraction is to be made with ammonia water then formula No. 7 is to be commended. The great increase in tinctorial power obtained by using a menstruum of proper alcoholic strength is proved, and if alcoholic extraction is to be the basis of the official formula, then formula No. 4 should be approved.

Formula No. 8 is based upon the principle of neutralizing the alkaline bases present in the cudbear and then extracting the liberated colorings with alcohol. The results are pleasing, the preparation is perfectly clear and keeps without change, which cannot be reported of the samples made by ammoniacal extraction, which after keeping for several months, show more or less tendency to precipitation. The product shades toward a brick-red on account of the free acid present, but on dilution gives a bright red to cherry-red. While the acidity might prove objectionable in some preparations, the results indicate a method of using cudbear to advantage in some solutions where the trace of acid is not contraindicated.

The Color Constituents of Cudbear.

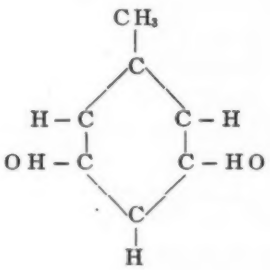
The text-books quite commonly are contented with the statement that the principal coloring substance present in Orchil and Cudbear is *Orcein.* Still more common is the misleading statement that *Orcin* in the presence of ammonia and air produces a colored substance *Orcein.* No mention is made of any other colors produced by such exposure or oxidation and so generally the value of cudbear as a dye has been attributed entirely to the orcein content. The attempt to standardize cudbear and its tincture have likewise, in a large measure, taken the direction of comparing these with standard solutions of orcein. The assumption here again was that orcein represented all that was valuable or essential in cudbear as a coloring for pharmaceutical preparations. If such assumption was correct then the problem of obtaining uniform colored solutions with cudbear could be readily solved by the use of solution of *Orcein.*

My experiments on the color constituents of cudbear were primarily undertaken with the thought of formulating a practical method of isolating the orcein in a state of sufficient purity to be used satisfactorily as a coloring in pharmacy. I have found it a most fascinating field of study, associated with peculiar and bewildering difficulties. The time at my command has not permitted of a thorough investigation of the colors isolated and the incompleteness

of the investigation is admitted. Some of the results and conclusions are presented in this communication. It will be impossible to give herein all of the methods tried and much of the details of the experimentation is necessarily omitted.

The studies of E. Bachman [6] indicate the complexity of the study of the lichen pigments. The numerous organic acids present in this family are in nature's laboratory largely converted into orcin. These acids have been the subject of elaborate investigations by many of the most prominent European chemists. The facility with which they form esters further complicates the study and must affect the colored products resulting from their oxidation and present in the commercial dyes.

Orcin is not only present in many of these lichens, but is increased in the process of manufacture of the dye by splitting up the organic acids and compounds by heating with alkali. It can be produced by a number of processes, and when pure appears in colorless, sweet tasting crystals containing water of crystallization and melting at 58° C. It is soluble in water, alcohol and ether.[12] Chemically, Orcin is dihydroxy methylbenzene or Dioxytoluol $C_6H_3(CH_3)(OH)_2 + H_2O$.



Its close relationship to benzene shows that nature had anticipated man in establishing in her laboratory dye factories that simulated the process of the aniline dye chemists. The manufacturers of the lichen dyes have produced the same coloring materials from natural sources that can be duplicated in the chemists' laboratory by modern synthetic methods from definite chemical compounds. A priori, one could readily predict that orcin would yield a number of substitution compounds besides *orcein.*

---

[12] *Schmidt Pharm. Chem.,* 1012.

Robiquet [13] was the first to observe that orcin in the presence of air and ammonia yielded a dye and named this body *Orcein.* This was further studied by Heeren [14] and Dumas [15] and Kane.[16] It was learned that orcein could be readily produced by exposing slightly moistened orcin in a watch crystal over a vessel containing strong solution of ammonia until it became brown and then dissolving this in water with the aid of a few drops of ammonia water and from this solution the orcein was precipitated by acetic acid.

From commercial orchil, orcin can be produced by moistening with hydrochloric acid, drying and then extracting with boiling alcohol. The alcohol is distilled off and the residue washed with water and then with ether when a carmine red powder remains.[17]

According to Liebermann [18] two dyes are produced by the action of air and ammonia on orcin, both being brown amorphous substances with green lustre. The one which is the chief product when the ammonia is in excess is the least soluble in alcohol and ammonia.

Especially worthy of note was the investigation of Zulkowski and Peters [19] who found that on allowing orcin to stand for two months over ammonia that three different dyes were produced: (1) Red orcein, which crystallized from hydro-alcoholic solution in microscopic crystals which yield a brown powder. The solution in alcohol is carmine red and with alkalies and alkaline carbonates gives a blue violet color. It is insoluble in water and ether, soluble in alcohol and acetic acid and acetone. (2) A yellow crystalline dye material which is soluble in warm water, alcohol and ether, giving a yellow colored solution. (3) A lackmus-like dye, amorphous and insoluble in alcohol.

These authors showed that these three bodies would be produced in three days by mixing 100 parts of crystallized orcin, 200 ammonia water (22 per cent. NH_3) and 1200 of hydrogen dioxide 3 per cent. The violet mass resulting is then acidified with hydrochloric acid,

[13] *Ann. Chem. and Phys.* (2), 47, 238.
[14] *Schweigger's Journ. of Chem.*, 59, p. 313.
[15] *Ann. Chem. Pharm.*, 27, 145.
[16] *Ann. Chem. Pharm.*, 39, 25.
[17] See *Die Chemie der Natürlichen Farbstoffe,* Dr. Hans Rupe, to which I am indebted for many of these references.
[18] *Ber. d. deutsch. Chem. Ges.*, 7, p. 247; 8, p. 1649.
[19] *Monatsh. f. Chem.*, 11. p. 227.

the precipitated Orcein is collected and washed with water until no longer acid and then dried at a moderate temperature. On concentrating the filtrate and wash water and adding sodium chloride a second precipitate of orcein can be obtained. On washing the orcein with ether the yellow dye is removed. The orcein is dissolved off the filter by boiling alcohol and there is left on the filter a lackmus-like dye material insoluble in alcohol. On evaporating the alcoholic solution of orcein it is obtained as brown crystalline powder. If water be added during the evaporation the orcein is left in glistening flakes.

The method of Zulkowski and Peters with ammonia and hydrogen dioxide was tried on a small scale, using Merck's orcin and the proportion of ingredients as stated. After allowing the mixture to stand for five days, the purple colored mass was acidified with hydrochloric acid and the precipitated orcein washed on to a filter with distilled water and the washing continued until the filtrate was free from acid. The filtrate was still of a bright red color and was washed with several portions of ether; the latter became a deep orange color and yielded on evaporation a reddish orange residue, and this was no doubt the yellow described by these authors. A small portion of this orange residue was soluble in chloroform and the chloroform was colored a distinct yellow, not orange, and the residue from evaporation of the chloroform was much lighter in color and had much less tinctorial power. This would indicate that the yellow of these authors was composed of two compounds, the one soluble in chloroform and the other scarcely soluble in chloroform, but very soluble in ether and present in relatively large amount.

The chloroform soluble yellow gave with ammonia a lilac-pink and with hydrochloric acid a light yellow coloration. With alcohol the solution was yellow. It dyed silk in ammoniacal bath only an indistinct faint light pink and in acid bath a maize yellow. For wool it had little affinity, and failed to dye in alkaline bath and in acid bath only a very pale yellow. The tinctorial value of this yellow was very slight. Owing to the small amount of material obtained the results were not satisfactory.

The orcin orange, the ether soluble yellow, in contradistinction gave orange colored solutions in water and alcohol. With ammonia it gave a bright violet color and hydrochloric acid changed this to

light scarlet-yellow. The tinctorial strength was very marked. In acid bath it dyed both silk and wool a deep rich mandarin orange and in ammoniacal solution silk was dyed a salmon pink.

The precipitated *orcein,* after washing free from acid, was dried on the filter and then washed with ether. The ether extracted considerable of the yellow dyes which had been carried down with the orcein and these reacted the same as that obtained from the wash water. The orcein was then extracted by washing the filter with hot alcohol and on evaporation the alcohol solution yielded a glistening residue which gave a red-brown powder. There still remained on the filter a very small amount of precipitate not soluble in the alcohol. This gave with alkalies a distinct litmus-like blue and with acids a red and corresponds to the "lackmus-like" dye reported by Zulkowski and Peters.

The orcein so obtained was almost insoluble in water and ether and with alcohol gave a deep carmine red-colored solution and in acetone a cherry red. With alkalies it gave a blue-purple and with hydrochloric acid a red coloration. It possessed strong tinctorial properties and dyed silk in acid bath Bordeaux red and in alkaline bath purple. It dyed wool in acid bath a brownish-red and in alkaline bath a violet blue.

The wash water from this experiment was still of a deep red color even after having twice precipitated the orcein and washing with ether to extract the yellows and showed that it still retained much coloring. It was washed with warm amyl alcohol, which removed nearly all of the coloring, leaving the wash water only slightly colored. The amyl alcohol solution was separated and washed with water to remove dissolved salts and then evaporated on a water bath. It yielded a copious reddish purple residue. This was soluble in water and more readily in alcohol, giving red-purple solutions. With ammonia the solution becomes intensely purple and with hydrochloric acid a reddish purple. It possessed powerful tinctorial value and dyed silk and wool, either in acid or in alkaline baths, beautiful shades of purple. This dye appears to have escaped the attention of other investigators, although present in considerable quantity, probably because they failed to examine the wash waters. Provisionally we will name it *Orcin Purple.*

In order to determine the conditions under which orcin purple is formed and whether the presence of hydrogen dioxide influenced its production a few test tube experiments were tried. It was

learned that orcin in the presence of potassium hydroxide and water yielded largely the yellow dye. In the presence of ammonia in excess and without any hydrogen dioxide being added and allowing the oxidation to proceed only two or three days and not to completion, all three dyes, red, yellow, and purple were produced, the yellow and especially the purple exceeding the amount of orcein. Moreover, that the purple dye was soluble in ether when in ammoniacal solution and could be partly recovered in this way even before acidifying and precipitating the red. Excess of ammonia and incomplete oxidation are conditions that are to be expected in the process of manufacturing cudbear. It will be thus seen that orcein is not the only product resulting from the oxidation of orcin. While the blue or lackmus-like dye and the yellow readily soluble in chloroform are present in such small quantity that they can probably be ignored, the orange is present in notable amount and modifies the color of the red with the production of brighter shades. Under certain conditions the orcin purple is produced in quantity sufficient to materially affect the color.

Before taking up the Zulkowski and Peters's paper and process for experimentation, the writer had tried a number of methods for separation of the color materials from cudbear and some of these merit recording. The original thought was to obtain the coloring by precipitation as a lake with a metallic salt. Lead subacetate was found to most completely precipitate the coloring.

Process A—50 gms. of cudbear, previously washed with 250 c.c. of cold water, was treated with successive portions of diluted ammonia water by maceration and the filtered solutions concentrated and solution of lead subacetate added so long as a precipitate was formed. The precipitate was washed by decantation and then on a filter until free from soluble lead salt. It was then suspended in water and a current of hydrogen sulphide passed through until the lead was entirely converted into sulphide. The mixture was then evaporated to dryness and the resulting mass powdered and divided into two equal portions. The one was extracted with warm alcohol and it was attempted to extract the other with diluted ammonia water. It was found, however, that the ammonia water softened the filter paper so that the lead sulphide passed through and even after repeated evaporations and resolution the lead sulphide persisted in the filtrate. The alcoholic solution from the other half was evaporated and yielded a red powder. The yield was exceedingly

small and the tediousness of this process precludes its recommendation. The residue gave a bright red solution in alcohol and with ammonia gave a purple coloration. The alcoholic solution had a peculiar fluorescence. To ether it yielded a yellow dye, and from the ether extract water removed traces of a substance which, on concentrating the aqueous solution and adding potassium hydroxide, gave a pale yellow iridescent liquid with pale green and pink shadings. This was attributed to traces of organic lichen acids and similar reaction was obtained from the washings of the cudbear and from other extractions.

Process B—50 gms. of cudbear was washed as previously directed, mixed with 10 c.c. hydrochloric acid, dried and then extracted for 12 hours in a Soxhlet apparatus with alcohol. The extraction even then was not complete. The alcohol was distilled off and the residue evaporated to dryness on the water bath and reduced to powder. It was then of a purple-brown color and weighed 8 gms., equivalent to 16 per cent. of the cudbear taken. This will be referred to as extract by acid alcoholic process. Five grammes was purified by washing with water and then with ether as recommended in the published process. The ether was colored yellow and on evaporation gave the orange-yellow dye. Associated with it was a small amount of a violet-red dye that was insoluble in chloroform.

The purified extract was now of a dull red powder and weighed 1.75 gms., it gave with alkalies the blue-purple coloration and dyed silk and wool the orcein shades. In accordance with the published statement it should be orcein. It was, however, far from pure and, although made by alcoholic extraction, it was no longer entirely soluble in alcohol; a portion dissolved readily in alcohol and a smaller amount was very scarcely soluble even in boiling alcohol. This portion dissolved readily in ammonia water, yielding a purple solution, but when reprecipitated by the addition of acid its solubility in alcohol, even in this freshly precipitated state, was not increased. This portion was likewise much weaker in tinctorial value.

Process C—50 gms. of cudbear was washed as in previous processes, then mixed with 25 gms. of ground cork and percolated with a mixture of ammonia water 1 volume, water 3 volumes, until 3500 c.c. of percolate was obtained and the cudbear was fairly well extracted, although not exhausted. The percolate was concentrated to 500 c.c. and sufficient hydrochloric acid (25 c.c.) was added to make the

solution decidedly acid, then warmed and set aside for 24 hours for the precipitate to settle. The precipitate was collected on a filter and washed till free from acid, then dried and powdered. It yielded a red-brown powder which gave a purple solution with alkalies, and dyed silk in acid bath a light purplish red, corresponding to what is listed by silk manufacturers as crushed strawberry, and in alkaline bath a red violet. This impure orcein we will designate as *Perseo Red.* It is composed of two red coloring substances, one soluble in alcohol with a carmine red solution, the other practically insoluble in alcohol but soluble in ammonia water, and the ammoniacal solution on evaporation yields a shining lustrous purple powder which is insoluble in alcohol, but soluble in diluted alcohol to deep purple red solution. The experience with these red dyes obtained in both processes B and C seems to confirm the statement of Liebermann [20] as to the existence of two red dyes produced by oxidation of orcin with ammonia and air.

The filtrate and wash water from the precipitated perseo red was concentrated and extracted with ether which extracted the yellow dye. Subsequent extraction with amyl alcohol yielded a brownish-red solution and on evaporating off the amyl alcohol there remained a lustrous brown powder. This was soluble in water and in alcohol yielding purple-red solutions which on the addition of ammonia became a brighter red but not purple. It dyed silk in acid bath a heliotrope and in alkaline bath an "old pink" shade.

In attempting to isolate the different coloring matters present in cudbear, either by precipitation or by the use of immiscible solvents, one must recognize the difficulty of their separation in an absolutely pure state. Traces of the associated colorings are almost sure to adhere and modify the shades produced in dyeing. Nevertheless, there are several well defined and distinct colors which are evidenced in every one of these methods and prove that in cudbear we have to deal with the orcin-like reds, a yellow and the purple in varying proportions. The samples of the isolated dyes, colored solutions and dyed silk and wool fibres demonstrate this.

In order to compare the strength of the red dyes obtained in the processes described they were compared with a sample of orcein as supplied by Merck & Co. This orcein was insoluble in water,

[20] *Ber. d. deutsch. Chem. Ges.*, 7, p. 247; 8, p. 1649.

chloroform, petroleum benzin and benzene. In alcohol it gave a carmine red solution and its solubility in alcohol was increased by the addition of a small amount of ammonia. With methyl alcohol it gave a carmine red solution, with acetone a scarlet, and amyl alcohol was colored deep pink. With ether it yielded a pale yellow solution, this filtered off gave with ammonia a violet and when acidified with hydrochloric acid was changed to a pink lilac color. It was noticed that on adding more ether, this at first remained colorless, but gradually became yellow as if some change was taking place in the presence of that solvent.

Solutions of each of these red dyes were prepared by using .050 gm. of the dye, 5 c.c. ammonia water and sufficient diluted alcohol to make 100 c.c. The Merck's sample was taken as the standard and for comparison 1 c.c. of its solution was diluted to 100 c.c. with distilled water. The results were as follows:

Merck's Orcein	Solution, clear, dark purple	1 to 100 c.c. Standard
A.—Red by Process A not purified beyond alcoholic extraction.	Solution, clear, lighter red-purple.	5 c.c. to 100 c.c.
B.—Purified red by acid extraction.	Solution, clear, purple.	2.25 c.c. to 100 c.c.
C.—Perseo Red.	Solution, clear, deep purple.	3 c.c. to 100 c.c.
D.—Orcein by Zulkowski and Peters' method.	Solution, clear, deep purple.	.8 c.c. to 100 c.c. Solution more blue as if trace of litmus present.

On acidifying dilutions of these solutions the red produced was similar to a litmus red; this was somewhat less marked in solution A. Aromatic elixir colored by these lacked the attractive brightness of cudbear coloring. It was concluded that orcein must have a limited field of usefulness in pharmacy and that it could not displace cudbear with satisfaction.

Extract of Cudbear.

Desiring to obtain a preparation that would represent all of the coloring present in cudbear, extracts were made. Preliminary experiments showed the necessity of washing the cudbear with cold

water to remove salts before extraction if non-hygroscopic and powdered extracts were to be expected and in these experiments, this was always a preparatory act. Alcohol, acetic acid, ammonia water U.S.P., and a mixture of ammonia water and water 3, were all used as menstruums as well as the acid-alcoholic hot extraction previously referred to. It was found that the resulting extracts could be dried and powdered, but that they were not freely soluble in alcohol or water, and that with each, complete solution could be effected by the addition of ammonia.

For the comparison of these extracts solutions were made by the following formula: Extract .5 Gm., Ammonia Water 5 c.c., Diluted Alcohol sufficient to make 100 c.c. The tabulated statement exhibits the results. Efforts to compare these with the Arny and Raubenheimer Standard Solutions were not satisfactory and comparison was made by using the dilution of the solution of the alcohol extract as a standard.

Menstruum	Yield	Character of Solution	Amount required Match Standard.
Alcohol	8.3%	Solution complete, sediment very little.	1 c.c. to 200 c.c. standard.
Acetic Acid	11.2%	Poorly soluble and solution not completely effected.	8 c.c.
Ammonia Water	13.0%	Solution complete.	3 c.c.
Ammonia Water 1 Water 3	20.0%	Solution nearly complete.	2.5 c.c.
HCl and Alcohol	16.0%	Solution nearly complete.	4 c.c.

Aromatic elixir colored by any of these solutions was distinctly purple but the red coloring was readily produced by neutralizing with citric acid. The use of an extract of cudbear would very materially reduce the variability in color of preparations in which it was used and it would also be available for the preparation of a more uniform tincture. Acetic acid extract is ruled out on account of its poor solubility and lack of strength. On the score of economy ammonia water extraction appeals but on the basis of strength and reliability alcoholic extraction is to be preferred.

LIME-WATER.

By Herbert J. Watson, P.D.[1]

Lime-water, officially known as Liquor Calcis, is a solution of Calcium Hydroxide. The United States Pharmacopœia describes lime water as, "A saturated aqueous solution, which should contain not less than 0.14 per cent. of pure Calcium Hydroxide [$Ca(OH_2)$–73.56." It also states, "The percentage of Calcium Hydroxide varies with the temperature at which the saturated solution is prepared, being about 0.17 per cent. at 15° C. (59° F.), the percentage diminishing as the temperature rises."

In the preparation, the lime is directed to be slaked and washed by the gradual addition of water, equal to one-ninth the volume of the finished solution. Agitating at intervals and decanting the supernatant liquid, which contains the impurities, follows the slaking and dilution with the water.

Recent experiments, in the preparation of lime water, that I have made, prove that the solution of Calcium Hydroxide (official strength) may be had within fifteen minutes, if the lime is slaked in boiling distilled water. Small quantities of the water added sufficient to keep it moist. Washed on a filter with boiling distilled water. The washing may be repeated once or twice with more of the hot water. The filter paper is then perforated at the bottom and the entire amount of the cold water (15° C. or 59° F.) is used to wash the Calcium Hydroxide off the filter paper in the bottle. After violent agitation with the cold water, the solution was filtered for testing and 50 c.c. of this solution required 22.1 c.c. $\frac{N}{10}$ H_2SO_4 V.S. to neutralize the Calcium Hydroxide present (U.S.P. requires not less than 19 c.c. of the $\frac{N}{10}$ H_2SO_4 V.S. to neutralize 50 c.c. of the lime-water).

The same lime, as used in the above experiment, was slaked at 75° F. and tested after 5 hours standing required but 20.5 c.c. $\frac{N}{10}$ H_2SO_4 V.S. This shows a gain of 1.6 c.c. in favor of slaking with the boiling water. The intense reaction, taking place during the slaking with the heated water gave a much finer powder than

[1] Read at the meeting of the Delaware Pharmaceutical Association, June, 1912.

with the water at room temperature. This fine powder gave a greater surface to the water, resulting in an increased solubility. The above solutions were agitated but once.

The prepared lime in small bottles sufficient to make one gallon Lime-water U.S.P., I found to make a very strong lime-water. At room temperature, the solutions prepared from this special lime, were stronger than lime-water prepared from solutions of the stone-lime in cold water. It required 24.1 c.c. $\frac{N}{10}$ H_2SO_4 V.S. for 50 c.c. of this specially prepared lime-water.

Lime-water prepared from imported lime C.P. made an excellent solution. This preparation required 22.2 c.c. $\frac{N}{10}$ H_2SO_4 V.S. to neutralize 50 c.c. of the water prepared at room temperature.

The solution of Calcium Hydroxide, at various temperatures, is very interesting. The mistaken idea, that we so often hear our fellow pharmacists say, "Lime-water made from tap water, is just as good or will do," will not stand. Lime-water, prepared from tap or hydrant water, is not the official Liquor Calcis U.S.P. The Pharmacopœia, distinctly and definitely, prescribes distilled water (15° C. or 59° F.) and not tap or hydrant water at the prevailing temperature.

Water taking up sufficient Calcium Hydroxide (at 15° C. or 59° F.) to require 24.1 c.c. $\frac{N}{10}$ H_2SO_4 V.S. Solution at room temperature (21.7° C. or 71° F.) required 21.7 c.c. $\frac{N}{10}$ H_2SO_4 V.S. to 50 c.c. of the lime-water. The decrease of the Calcium Hydroxide is very small when the temperature rises over 71° F. Water at 100° F. was used for this preparation, and 21.4 c.c. $\frac{N}{10}$ H_2SO_4 V.S. were required for 50 c.c., resulting in .3 c.c. of a decrease under that prepared at room temperature.

The solution deteriorates rapidly upon exposure to the ordinary atmosphere. Even with electric lighting in the place of illuminating gas, the decomposition is rapid. Experiments show that Calcium Hydroxide in solution is reduced from 2 to 3 per cent. a day, when it is left unstoppered. In one solution, the lime-water was reduced from a 100 to a 21 per cent. solution in 8 days. In the presence of lime and the bottle left unstoppered, the lime-water was reduced to an 81 per cent. solution of Calcium Hydroxide in 6 days. The reduction of the lime-water, in this last instance, is followed by the carbonation of the lime and the entire mass is rendered inert.

I found one of the samples that I collected was undoubtedly due to this mistake of slaking the lime in a large earthenware jar with a loose lid. They permitted "the water to soak and thoroughly be saturated with the lime," as they termed it. This sample was about 13 per cent. strength.

The majority of the samples examined were identical in strength with those prepared with tap water. Few were strong, indicating special care in preparation from selected lime. With few exceptions the examination of the specimens throughout the State was very satisfactory. Very few of the samples taken were found below strength.

I expected, according to a previous paper to this Association on Lime-water, to find at least every other specimen below strength, but it was a pleasant surprise when I tested so many before detecting a sample that was not full strength.

A RAPID ACCURATE METHOD FOR THE QUANTITATIVE ESTIMATION OF CHLOROFORM IN CHLOROFORM LINIMENT.

By Joseph L. Mayer.

A member of the Revision Committee of the Pharmacopœia, recently called my attention to the advantage of making official a method for the quantitative determination of chloroform in chloroform liniment, and the lack of a published process for the same.

The subject being an important one, I began experimenting with the object in view of evolving a method whereby the pharmacist could easily and accurately make the estimation.

A method, which at first gave promise of yielding satisfactory results, was to precipitate the chloroform out of the liniment by means of 10 per cent. ammonium hydroxide and while the results obtained were satisfactory when the sample was of U.S.P. strength, when the quantity of chloroform contained in the liniment was less than 25 per cent. or more than 30 per cent., the results were too far from the truth to be of value. The method was therefore abandoned. An effort to throw out the chloroform by means of centrifugal force did not yield concordant results.

It soon became apparent that the soap in the liniment was the

disturbing factor, and that to obtain satisfactory results it was necessary to distill the chloroform. Remembering this fact, the following method was devised:

Into a test-tube having a capacity of about 85 c.c. and about 25 mm. in diameter, place 10 c.c. of distilled water and 10 c.c. of liniment to be analyzed, accurately measured with a pipette; to prevent bumping, a small piece of pumice stone which has previously been heated to white heat and thrown into water is added. The test-tube is connected with a Liebig condenser by means of corks and bent tube. For a receiver use an accurate 25 c.c. cylinder graduated in tenths or fifths of a c.c. containing 5 c.c. distilled water. It is not necessary to have the condenser tube come in contact with the water. All that is required is to have it project into the cylinder. By means of a small naked flame, quietly distil the chloroform into the water contained in the cylinder. It is easy to know when the chloroform is all distilled by watching the receiving cylinder. As the chloroform distils it sinks to the bottom then comes a lighter distillate which remains on top and is perfectly clear and then a distillate which forms a milky layer occupying about 1 c.c.; after this turbid zone has appeared remove cylinder; stopper it with a sound cork and mix by shaking thoroughly; then remove the cork and add diluted sulphuric acid (10 per cent.) to the 25 c.c. mark and shake thoroughly. In a few moments the chloroform will have settled to the bottom in a clear layer and all that remains is to multiply the c.c. of chloroform by 10 to obtain the percentage of chloroform in the sample. The entire operation does not require over fifteen minutes.

The results obtained on a large number of samples of known but varying strengths, proved the method to yield such very accurate results, that should the Revision Committee decide to make official a method for the quantitative estimation of chloroform in chloroform liniment, it is suggested that they adopt this one.

Of course a description of the method for use in the Pharmacopœia could be very much shortened as I have purposely gone into detail in describing it.

In view of the accuracy of results, ease of application, and simplicity of apparatus, the method has everything to commend it.

I would take this opportunity of acknowledging my indebtedness to my assistant, Mr. I. Schwartz, for valuable aid rendered in connection with the work.

WEIGHTS AND MEASURES SHOULD BE GUARANTEED U.S.P. STANDARD.[1]

By Joseph W. England.

There is probably no more important need in the pharmaceutical world than the necessity of having accurate and uniform weights and measures, more especially measures of volume.

It is simply idle to standardize the potent remedies of the Pharmacopœia, with the greatest possible degree of accuracy, and then measure them with measures that are not accurately graduated.

As an illustration, three dozen 8 ounce graduates were purchased by Whitall, Tatum Co. in widely separated localities and compared with the standards used in their factory. The results were startling. They were as follows:

Not *one* of the 36 graduated measures was accurately graduated.

Some were better than others, but all were bad.

On one graduate, the 6 ounce mark was correct, but all the remaining marks were wrong.

In one lot of twelve graduates, 6 fluid drachms of liquid were required to reach the graduation marked ½ fluidounce, a variation of 50 per cent!

All graduates should be guaranteed by manufacturers, and marked:

"Guaranteed U.S.P. Standard by ——;" and pharmacists should buy no other. *It is fully as important that weights and measures be guaranteed to be of U.S.P. Standard as it is of drugs.*

A standard graduate should be made of good flint glass properly annealed and graduated, the usual types being conical, narrow cylindrical, and broad cylindrical. The best forms are of blown glass, not pressed glass. The annealing of the glass is done in lehrs or tempering ovens 75 to 100 feet long. The graduates go in at one end red hot, and in twelve hours come out at the other end cold, and properly annealed. It has been alleged that graduates may be tempered by placing them in cold water, bringing the water to the boiling point and cooling. But glass workers claim that such a method is of no practical value, as the temperature of the boiling

[1] Read before the Pennsylvania Pharmaceutical Association, June, 1912.

water is not high enough to permit any great readjustment of the relative positions of the various molecules of the glass.

The standard followed by Whitall, Tatum and Co., is 1 fluid-ounce = 29.5161 grammes of water when weighed in dry air at a temperature of 15° C., barometric pressure of 760 mm., the coefficient of expansion of the glass being assumed to be 0.000025 and the density of the brass weights 8.3. These figures are derived from the original data in use at the National Bureau of Standards of the United States, Washington, D. C., and the calculations are carried to any number of decimals necessary in the case of each instrument. The best method of graduation is that in which the graduates *deliver* the quantities indicated, every line in each graduate being determined by actual measurement. Where mechanical division is employed, the graduates will vary in delivery.

There should be no variation in the delivery graduates, other than that caused by the personal factor. In measuring liquids, the lower meniscus should be, of course, always observed; if the graduate is not held in a perfectly level position, more or less of the liquid will be measured, according as the graduate is tilted backward or forward.

Personal accuracy in the reading of graduates depends somewhat upon the diameter of the container at the point where the reading is taken. It is for this reason that the narrow cylinder is apt to yield more accurate results than the cones, and is preferred in the analytical laboratory; the cone is sufficiently accurate, however, for galenical work, and is more easily cleaned out.

The weights and measures recognized by the U. S. Pharmacopœia are derived from or based upon those of the metric system, as the United States National Prototype Standards of the Meter and Kilogramme in the custody of the National Bureau of Standards at Washington, D. C., and the system of Apothecaries' Weights and Fluid Measures are used almost wholly by the physicians of this country in prescribing and the pharmacists in dispensing. The use of the metric weights and measures is exceedingly limited, but it is growing.

According to the U.S.P. (VIII) P. LIII, the standard temperature for the solubility of substances in liquids, for taking specific gravity and for volumetric operations in the Pharmacopœia is 25° C. (77° F.); in the former revision it was 15° C. (59° F.). This change has been made on account of its greater convenience, and

because it suited the greatest number of laboratory experts and pharmacists in the United States. In the case of alcohol and wine, however, the temperature of 60° F. (15.667° C.) was recognized, for the present, since all the laws and regulations of the United States, referring to alcohol and alcoholic liquids in general, are still based on this degree of temperature.

The standard temperature used by the glass manufacturer for graduating his measures is still 15° C., although it was changed in the eighth revision of the Pharmacopœia, from 15° C. to 25° C., as it will be probably in the ninth revision; but this error will be doubtless corrected by the manufacturers as soon as the temperature for the ninth revision has been decided upon.

The standard fluidounce of water used by the glass manufacturer in graduating his measures is: 1 fluidounce = 29.5161 grammes at 15° C. The standard of the U.S.P. (VIII) is 1 fluidounce = 29.5737 grammes at the maximum density of water (4° C.) in vacuo. This is a very small difference, when the expansion of the water from 4° C. to 15° C. is considered; and proper adjustments will doubtless be made as soon as the standards of the ninth revision become official.

The use of graduated prescription bottles should be discouraged. They vary greatly in accuracy, and their use is a delusion and a snare. They are blown in moulds, and vary in contents according to distribution of the glass in the mould. Sometimes this is more uniform, and sometimes less, and hence the quantities marked on such containers must be inaccurate. There is no substitute in prescription work for an accurately graduated measure.

In conclusion, it should be added that the American-made glass graduates, in accuracy and appearance, are superior to those made abroad, and much more likely to accord with the U.S.P. standard.

PHILADELPHIA COLLEGE OF PHARMACY, QUARTERLY MEETING.

The quarterly meeting of the Philadelphia College of Pharmacy was held June 24, 1912, at 4 P.M., in the Library. In the absence of the President, Howard B. French (who was in attendance as a delegate to the Republican National Convention at Chicago), Mr. George M. Beringer was called to the chair.

The minutes of the Annual Meeting held March 25th were

read and approved. The minutes of the Board of Trustees for March 5th, April 2d, May 7th and 14th were read by the Registrar, J. S. Beetem, and approved.

Report of Committee on Membership: In the absence of the Chairman, Professor Charles H. LaWall, the report was read by J. S. Beetem. The report gives the number of Active, Associate, and Honorary members, makes some suggestions concerning delinquent members, and of measures to interest others to become members. The report was discussed by Messrs. Lowe, Beetem, Beringer, Stroup, Kraemer, Poley and Weidemann, when the recommendations were approved.

Committee on Necrology reported that during the year two active members, Doctor George R. Vernon and Clemmons Parrish, and one honorary member, Doctor Walter Wyman, had died.

Report of delegates to the New Jersey Pharmaceutical Association, presented by George M. Beringer. The meeting of the Association was held at Atlantic City, June 4–7. The delegates from the College were cordially received. The attendance this year was not as large as last year, as the subject of Legislation did not occupy so much attention. The program contained a number of interesting and instructive events. An illustrated lecture by F. B. Kilmer on the Collecting and Marketing of Crude Drugs; papers were read by Dr. C. B. Lowe, Prof. Charles H. LaWall, E. Fullerton Cook, Charles Barrett and George M. Beringer; Prof. Joseph P. Remington made a very interesting talk upon the progress made in the 9th Revision of the United States Pharmacopœia. The Travelling Men's Auxiliary arranged for the entertainment of the Association a German Saengerfest and dance, which was greatly enjoyed. This Association also has a progressive Ladies' Auxiliary Association. The meetings of the New Jersey Pharmaceutical Association are attended by delegates from the near-by Colleges of Pharmacy, and the fact that many of the members who are active in the work of Association are graduates of our College always insures for the delegates a hearty and cordial welcome.

In connection with this report Mr. Beringer said a wider publicity should be given about the Edward T. Dobbins scholarship (available to a student from New Jersey), and suggested that the Secretary of the College forward the necessary information to the Secretary of the New Jersey Pharmaceutical Association for publication in the Annual Proceedings. The suggestion was approved.

Historical Committee.—Mr. Beringer, for the Committee, said there had been no report prepared, as the work of the Committee was now very largely taken up by the Permanent Committee on Centenary and History of the College, and in view of this suggested that the two committees be merged until the Centenary Celebration, when on motion of Professor Kraemer the suggestion was agreed to.

Professor Kraemer presented for the Historical Collection some drawings, made by Mr. Ray H. Machesny and others of the graduating class, that had been used in the preparation of this year's Class Book. They showed sufficient merit and were considered worthy of preservation.

Report of delegates to the Pennsylvania Pharmaceutical Association, by Prof. C. B. Lowe. The 35th annual meeting was held at Buena Vista, Franklin County, Pa., on June 18, 19 and 20. The attendance was excellent. The first meeting was held on Tuesday morning, reports of officers and committees being in order. The formal opening being in the evening when President Lemberger made his address. The Committee on Papers and Queries, Prof. E. P. Stroup, Chairman, presented 37 papers, all of them interesting and some of them quite important. The members of the faculty and graduates of the College being largely represented among the authors. The prize of $20 in gold for the best paper presented at the previous meeting was awarded to Prof. Henry Kraemer for his paper on "Variations in the Forms of Vegetables." (See this JOURNAL, 1911, p. 365.) Great interest was taken in the proposed Pharmacy law, much opposition was manifested by many of the country members, but after being modified to a considerable extent it was adopted, and ordered to be introduced at the next meeting of the Legislature. The social features were up to the former high standards of the Association. A most unique affair was presented, entitled "A Night in Japan," which was a great success. The next place of meeting is Forrest Inn, Pike County. The new President is Prof. Louis Saalbach, of Pittsburgh.

Professor Kraemer proposed a candidate for honorary membership, which, according to the rules, lays over for action till the next meeting of the College. The President made the following appointments: Delegates to the Sixtieth Annual Convention of the American Pharmaceutical Association, Prof. Joseph P. Remington, Chairman, Prof. Henry Kraemer, Prof. C. B. Lowe, Adolph W. Miller, M.D., and George M. Beringer.

Committee on Nomination.—Prof. F. X. Moerk, Chairman, William E. Lee, William McIntyre, C. Mahlon Kline, and F. P. Stroup.

C. A. WEIDEMANN, M.D.,
Recording Secretary.

ABSTRACTS FROM THE MINUTES OF THE BOARD OF TRUSTEES.

March 5th. Eighteen members were present.

Committee on Property reported that a revision of Insurance rates had been effected which would make quite a saving for the College.

Committee on Announcement reported the issuing of the February Bulletin and that the April number of the Bulletin was in course of preparation.

Committee on Instruction reported further in the matter of a Special Bulletin. In view of the detail work necessary in this connection a special committee of three was appointed to assist, viz.: Prof. S. P. Sadtler, Joseph W. England and George M. Beringer.

Mr. England gave some information concerning the establishing of the Pacific Coast Scholarship in the Philadelphia College of Pharmacy. An amount of money has been placed in the treasury of the Alumni Association as a nucleus for this purpose, but no further additions have been made to the fund. Mr. England stated he would communicate with the Pacific Coast Graduates and suggest their proceeding with the work of completing the Scholarship.

Mr. England read a communication from Professor Wulling of the Department of Pharmacy, University of Minnesota, relative to the loss of their building by fire. On motion, the sympathy of the Board in the loss they had sustained, was extended to them.

A vote of thanks was tendered William E. Weiss, class of 1896, for the donation of a projecting lantern.

A vote of congratulations was extended the Philadelphia Academy of Natural Sciences on their Centenary Anniversary.

April 2nd. Fourteen members were present.

A communication from the Secretary of the College was read, announcing the names of those who have been elected to membership in the Board of Trustees at the meeting of the College held March 25, 1912. Mr. George M. Beringer was elected Chairman of the Board, Walter A. Rumsey, Vice-Chairman, and Mr. Jacob S. Beetem was re-elected Registrar.

Committee on Library reported additional accessions to the Library by purchase and gifts. Number of persons using the Library in February was 159, in March 117.

Committee on Examinations reported that Edward Francis Kenney and Charles Louis Wagner had complied with all the requirements of the College for the Certificate of Proficiency in Chemistry and were now entitled to receive the same. It was so ordered.

Committee on Announcement reported that the Special Committee had given much attention to the preparation of the Special Bulletin, and when completed it would be very helpful to applicants preparing for entrance examinations. The Special Committee considering the suggestion of a preparatory or coaching school reported that the Board of Education would willingly co-operate by making arrangements for applicants of the different institutions to attend an evening High School class and thus assist them to take the entrance examinations or to remove conditions.

The Chair announced the Standing Committees for the year.

The Chair read a communication from President Vincent and Professor Wulling, of the University of Minnesota, expressing appreciation of the vote of sympathy extended by the Board.

May 7th. Seventeen members were present.

Committee on Library reported 275 books accessioned and classified during April. Several additions by gifts and that the Library had been used during the month by 75 persons.

Committee on Instruction presented a lengthy report including the annual reports of the Faculty and the recommendations of the Committee. The various recommendations were fully discussed and several were referred to the Committee on Property, the others being adopted.

May 14th. Seventeen members were present.

Committee on Examinations reported the names of those who had complied with all the requirements of the College for the Degree of Doctor in Pharmacy, Pharmaceutical Chemist, and for the Certificate of Proficiency in Chemistry. After a ballot was taken they were declared entitled to receive the same.

The Committee also presented the list of those entitled to receive prizes and the Chair announced the names of those appointed to present the same. The Committee also referred to the Wellcome Cup which had been given to the Second Year Class several years

ago, but which had never been formally presented. The Committee felt this award should be made the coming Commencement and that the rules and conditions governing the competing for the Cup by the Second Year Class should be the same as those governing the award of the President's Cup to the Third Year Class.

AMERICAN PHARMACEUTICAL ASSOCIATION.

COMMITTEE ON WEIGHTS AND MEASURES.

To the members of the American Pharmaceutical Association:—The Committee on weights and measures has we believe, usually confined its attention in an annual report to the status and use of the metric system. While there are still those who oppose the use of the Metric System we find that it is coming into more and more general use. For instance in the Aëroplane races at Belmont Park, L. I., the International Trophy Course was marked out in Kilometers. Furthermore in the machine shops nuts and bolts are being made according to Metric Measurement.

Your Chairman desired, in connection with the report presented last year to have some work done by the members of the Committee with a view of determining the accuracy of weights and measures actually employed in practice. Nothing was done by the Committee, but during the past year there has been considerable interest manifest, notably in New York, Pennsylvania and New Jersey, and some Legislation enacted tending to insure the consumer in obtaining correct weights and measures from the seller. The Bureaus of Municipal Research are devising plans for the creation of definite standards and for the appointment of responsible officials to protect the Public. I have had some correspondence with Mr. B. S. Thorp, the Philadelphia Assistant Manager for Whitall, Tatum and Company in regard to the subject of graduates used by druggists, and asked him to outline in condensed form the results of their observations on this subject to be published in connection with this report. Mr. Thorp writes as follows:

In an issue of "*Collier's Weekly*," published as near as we can recall, early in March, there appeared an illustrated article calling attention to the alarming prevalence in New York City, of the use of incorrect Weights and Measures in almost all lines of trade. The statement was made that the City of New York, through its Commissioner of Weights and Measures, had confiscated thousands

of false Weights and Measures, which were in daily use in all parts of the City, and, loading them on vessels, took them to sea and threw them overboard. The statement thus made confirmed our knowledge of the situation, particularly as applying to Glass Graduate Measures offered to the Drug Trade. We had previously purchased of three Wholesale Druggists, in widely separated localities, 3 doz. 8 oz. Graduates, for the purpose of comparison with those graduated correctly according to the Government Standards. The result of our examination showed indifference to the requirements of the U. S. Bureau of Standards, and proved conclusively that quality had been sacrificed to price. The manufacturers have undoubtedly been enabled to continue marketing these inaccurate Graduates by reason of the fact that many Druggists were not aware of the character of the goods being offered them.

Not ONE of the 36 Graduates was correct. Some were better than others, but ALL were BAD.

On one, the 6 oz. mark was correct, ALL THE REMAINING GRADUATIONS WERE WRONG.

In one lot of twelve, 6 drams of liquid was required to reach the graduation marked ½ oz.

In a recent letter received by us from Lucius P. Brown, Esq., Commissioner and Chief Chemist in the Office of the Pure Food and Drug Inspection Department of the State of Tennessee, he wrote us as follows: "The findings of this inspection have led to the belief that the failure of many of the drug samples to comply with the standards may be traced to the use of inaccurate or unsuitable Weights and Measures by the Retail Druggists. We have commenced a campaign in which every effort will be made to see that this condition is corrected." It is our belief that the findings of the Tennessee Inspection will be duplicated in many other localities and Druggists will be brought to realize that their best interests are conserved by using only those utensils which are of known accuracy.

We believe that this exceedingly valuable information ought to be in the hands of the Pharmacists generally.

HENRY KRAEMER, *Chairman.*

August, 1911.

WILLIAM TRELEASE AND THE MISSOURI BOTANICAL GARDEN.

Dr. William Trelease, director of Shaw's Garden since the death of its founder, Henry Shaw, in 1889, has tendered his resignation to the Board of Trustees. Dr. Trelease gives as his reason for his action the need of more leisure for scientific research and proposes to spend the next year or two abroad. The resignation was received with regret by the board. Dr. George T. Moore has been appointed as his successor.

Dr. Trelease was selected for the directorship more than twenty-two years ago by Henry Shaw, the creator of the Missouri Botanical Garden, commonly known by his name. The famed botanist, Prof. Asa Gray of Harvard University, was responsible for bringing Dr. Trelease to the attention of Mr. Shaw. Dr. Trelease came to St. Louis from the University of Wisconsin in 1885, where he had been professor of botany. He has always been active in every movement for the betterment of St. Louis, especially in the lines of his particular interests, and Shaw's Garden has come to stand for a force in such movements.

Since 1889 the average yearly cost of maintaining the grounds and plant houses has been about $25,000, aside from improvements. The average expenditure on the library, including purchases, binding and salaries of employees, has been about $4,700, and on the herbarium about $2,700.

The inventory of Henry Shaw's estate in 1889 showed that the herbarium then contained 67,554 unmounted specimens, appraised at $3,378, and 34,660 duplicates worth $1,733. At the end of 1911 it contained 698,706 mounted specimens, appraised at $104,805, about $40,000 more than the total expenditure on it for the twenty-two years,—and a very large number of exchange duplicates.

The library was inventoried in 1889 as containing 1,077 books and an unspecified number of pamphlets, probably about 2,000, as the ratio has since run, valued at $3,050. It now contains 28,428 books, 40,375 pamphlets and 112 manuscripts, valued at $107,417, and 792,967 index cards valued at $7,929.67, which exceeds by about $15,000 the total expenditure for purchases, binding and maintenance. The increase in 1911 alone equals the total original value. The library is well balanced in all departments of botany.

The formation of a library and herbarium unexcelled in the United States in their field, by expending something less than three-fourths of their appraised value for purchases, care and attendance, has been made possible through large gifts like the Engelmann herbarium and the Sturtevant library, to which a research establishment appeals. It rests equally on the exchange value of such a scientific publication as the Garden Report, confined to the results of original investigation and at once giving to the scientific world the fruit of its activities and forming a basis of extensive exchanges,—which bring nearly 1,500 distinct publications to the library and make the institution known and its output useful in the more than 1,000 scientific libraries all over the world from which exchanges come.

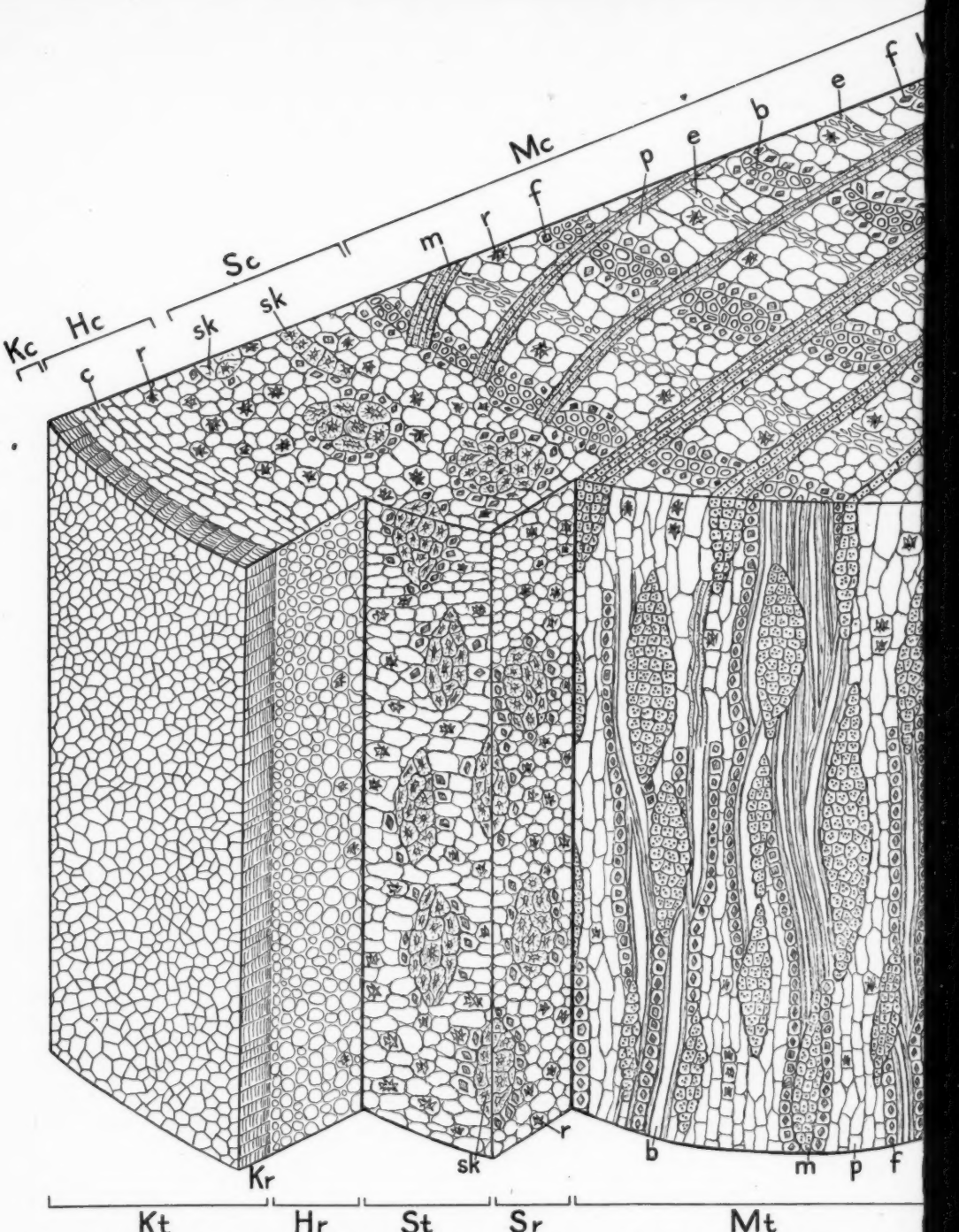
The inventory of the estate in 1889 includes about 12,000 plants which are believed to have represented not over 3,000 kinds. At the end of 1911 there were cultivated 12,668 different kinds, represented by possibly 60,000 specimens. 594 new kinds were added last year and over 85,000 specimens were grown and largely used for decorative planting.

Visitors to the Garden have been counted at the gate since 1898 only, when they were believed to be considerably more numerous than prior to 1889. At the end of 1908 the number of week-day visitors was found to have doubled in the decade, largely through the establishment of a tented chrysanthemum show each fall, and apart from this to have increased about one-half, the population of the city having increased about half as rapidly.

The laboratory equipment of the establishment, acquired for the most part during the last three years and devoted exclusively to scientific investigation, is now as unsurpassed in this country for work in the diseases of plants, the physiology of soil organisms and similar subjects as the herbarium is for systematic studies, and work is being done on these subjects by an unusually able if small corps of research fellows under the guidance of an expert, Prof. George T. Moore.

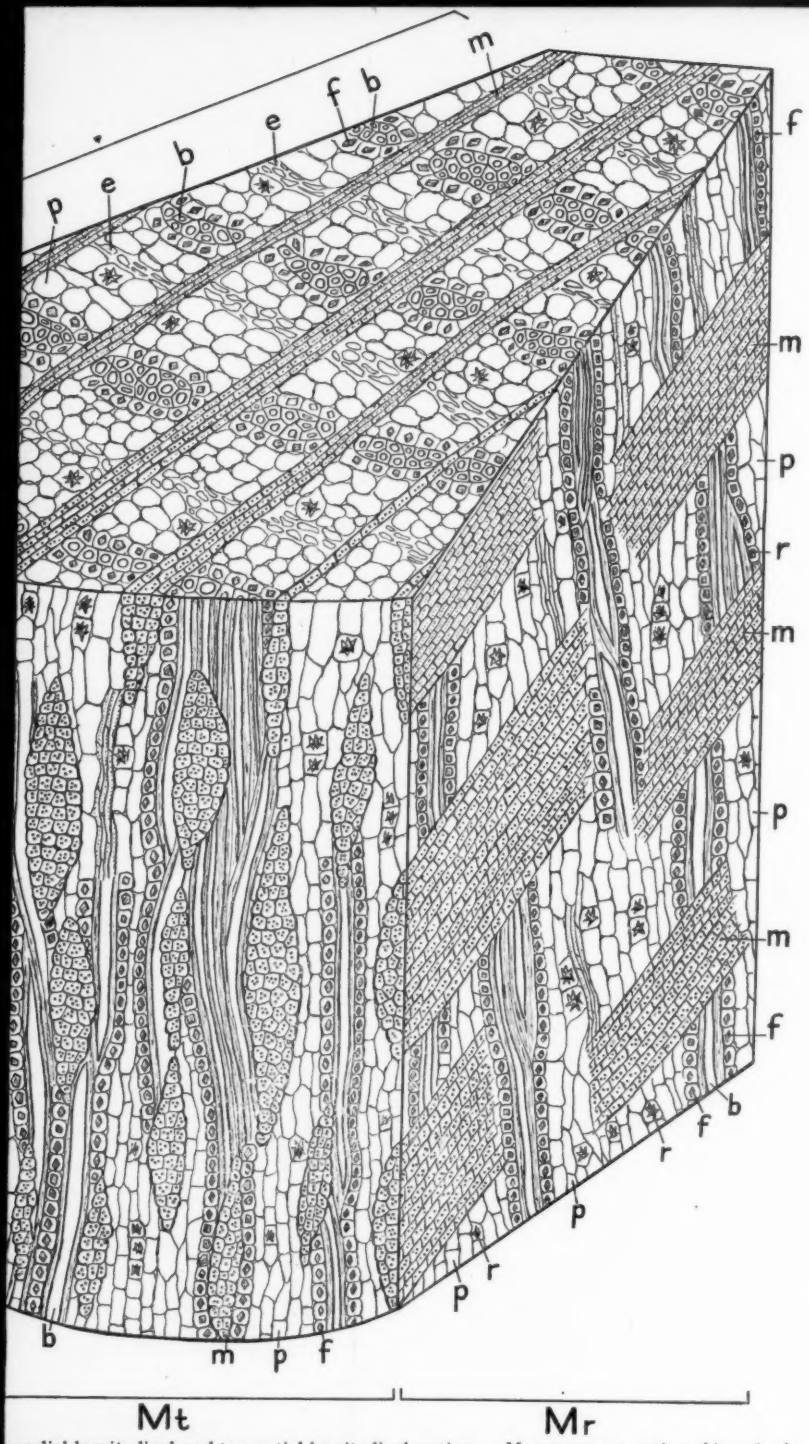
H. K.

Microfilm



The outer bark and part of the inner bark of Rhamnus Purshianus in transverse, radial-longitudinal, and tangential-longit
Mt, tangential-longitudinal section of inner bark; Mr, radial-longitudinal section of inner bark; Sc, transverse section of ston
cell area; Sr, radial-longitudinal section of stone cell area; Hc, transverse section of outer layers of cortex; Hr, radial-longitudin
verse, tangential-longitudinal, and radial-longitudinal sections of cork. b, bast fibers; f, crystal fibers; p, parenchyma; e, sieve;

ential-longit
tion of ston
l-longitudin
a; e, sieve;



, radial-longitudinal, and tangential-longitudinal sections. Mc, transverse section of innerbark; inner bark; Sc, transverse section of stone cell area; St, tangential-longitudinal section of stone uter layers of cortex; Hr, radial-longitudinal section of outer layers of cortex; Kc, Kt, Kr, trans- f, crystal fibers; p, parenchyma; e, sieve; sk, stone cells; m, medullary ray cells; c, collenchyma.

UofM